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Decompression induced bubble dynamics on ex vivo fat and muscle tissue surfaces with a new experimental set up

Virginie Papadopoulou[a,b,*], Sotiris Evgenidis[c], Robert J. Eckersley[d], Thodoris Mesimeris[e], Costantino Balestra[b,f], Margaritis Kostoglou[c], Meng-Xing Tang[a], Thodoris D. Karapantsios[c,**]

[a] Department of Bioengineering, Imperial College London, London, UK
[b] Environmental & Occupational Physiology Lab., Haute Ecole Paul Henri Spaak, Brussels, Belgium
[c] Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece
[d] Imaging Sciences & Biomedical Engineering Division, King's College London, London, UK
[e] Hyperbaric Department, St. Paul General Hospital of Thessaloniki, Thessaloniki, Greece
[f] DAN Europe Research Division, Belgium

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ABSTRACT

Vascular gas bubbles are routinely observed after scuba dives using ultrasound imaging, however the precise formation mechanism and site of these bubbles are still debated and growth from decompression in vivo has not been extensively studied, due in part to imaging difficulties. An experimental set-up was developed for optical recording of bubble growth and density on tissue surface area during hyperbaric decompression. Muscle and fat tissues (rabbits, ex vivo) were covered with nitrogen saturated distilled water and decompression experiments performed, from 3 to 0 bar, at a rate of 1 bar/min. Pictures were automatically acquired every 5 s from the start of the decompression for 1 h with a resolution of 1.75 μm. A custom MatLab analysis code implementing a circular Hough transform was written and shown to be able to track bubble growth sequences including bubble center, radius, contact line and contact angles over time. Bubble density, nucleation threshold and detachment size, as well as coalescence behavior, were shown significantly different for muscle and fat tissues surfaces, whereas growth rates after a critical size were governed by diffusion as expected. Heterogeneous nucleation was observed from preferential sites on the tissue substrate, where the bubbles grow, detach and new bubbles form in turn. No new nucleation sites were observed after the first 10 min post decompression start so bubble density did not vary after this point in the experiment. In addition, a competition for dissolved gas between adjacent multiple bubbles was demonstrated in increased delay times as well as slower growth rates for non-isolated bubbles.

1. Introduction

Decompression sickness (DCS) is a pathophysiology caused by gas bubbles which grow in the body during a reduction in ambient pressure (decompression) that can affect divers, astronauts, pilots and compressed air workers [1]. With over 7 million active recreational scuba divers worldwide [2,3] and new evidence for potential long term effects even in recreational divers who never had symptoms [4], it is alarming to note that scuba divers who follow current decompression procedures can still get DCS. Vascular gas bubbles are routinely observed even after asymptomatic SCUBA dives using ultrasound imaging and are considered a key element in the potential development of decompression sickness. The precise formation mechanism and site of these bubbles are still debated [5] and bubble growth from decompression in vivo has not been extensively studied, due in part to imaging difficulties [6].

Previous experiments on decompressed rats [7–10] looked at bubble growth/shrinkage on muscle and fat depending on the gas breathed. However, the main difficulty to date has been that the set-ups do not allow for real time observation of bubble growth during the decompression and tissues/animals have to be taken out of the chamber to be observed. For animals, the difficulty in locating bubbles means that bubbles often have to be injected [10]. For tissues, in addition to the potential problem from dislodgment

* Corresponding author at: Department of Bioengineering, Imperial College London, London, UK. Tel.: (+44) (0)20 75 94 36 64.
** Co-corresponding author at: Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece.
E-mail addresses: virginie.papadopoulou07@imperial.ac.uk (V. Papadopoulou), karapant@chem.auth.gr (T.D. Karapantsios).

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of bubbles with movement (opening chamber and taking samples out), the observation is then often done in non-ideal conditions such as waiting for the bubbles to float [11–13].

The first aim of this study is thus to develop a new experimental set-up to allow for the first time real time observation *during* decompression of bubble growth from desorption of inert gases out of solutions on tissue substrates. In order to quantify the different parameters that could influence inception delay times, growth rate, detachment and multiple bubble behavior, this set-up should allow for different gases and liquid compositions, temperature control, as well as optical recording of both bubble density per unit surface and precise bubble growth rate.

Higher subject fat percentage has been demonstrated as a risk factor for higher bubble grades post dive [14,15]. Although not fully understood, adiposity as a risk factor for developing decompression sickness (DCS) has been discussed for decades. It is usually attributed to either nitrogen solubility and diffusion arguments, or fitness-related variability in subjects' ability to cope with bubbles. We hypothesize that, in addition to nitrogen uptake and subject cardiovascular fitness, the hydrophobicity of adipose tissues may facilitate bubble growth during decompression.

The second aim of this study is therefore to investigate the role of the tissue substrate (surface), mainly of fat and muscle ex vivo rabbit tissues since fat and muscle have been used most in newer decompression modeling efforts [16–19], in the decompression induced bubble growth rate and density with nitrogen taken as the inert gas.

2. Material and methods

2.1. Experimental set-up developed

Figs. 1 and 2 show the different components of the set-up which allows for the first time the observation in real time during decompression of the growth rate of selected bubbles, but also bubble density per unit surface area, comparing how these vary for different tissue surfaces, decompression profiles, gas saturated liquid composition and temperature. Two configurations for optical acquisition were optimized to allow for subsequent semi-automated data analysis using image processing techniques: for growth data of individual bubbles maximum magnification and lighting straight from the back of the chamber, and for density data with lesser magnification and acquisition at an angle to look at a number of bubbles per surface area (calibrated with millimeter paper to get field of view precisely). The resolution of the optical system was assessed from calibration against a known thickness wire of 56 μm.

2.2. Experimental procedure

After Ethics Committee approval (59001/536) in accordance to EU Directive 2010/63/EU for animal experiments, ex vivo muscle and fat tissues from rabbits were obtained for the experiments and all animal handling was done by our collaborators in the veterinary school (see acknowledgements). Rabbits (male, 3 months old, $n = 8$) were anaesthetized then euthanized by injection of sodium pentothal and potassium chloride respectively. Excised dorsal subcutaneous white adipose tissue and quadriceps muscle samples were then extracted within 15 min and placed directly in physiological saline. These were stored at 4 °C for preservation and used in the experiments within 48 h. The tissues were covered with nitrogen saturated distilled water and eight decompression experiments performed, from 3 bar to 0 bar, at a decompression rate of 1 bar/min as described thereafter.

The following description relates to our particular experiment, however different gases, liquids, tissues and temperatures could be used with this set-up. A compressed nitrogen tank is used to saturate distilled water at just above 3 bar (taking reference of 0 bar

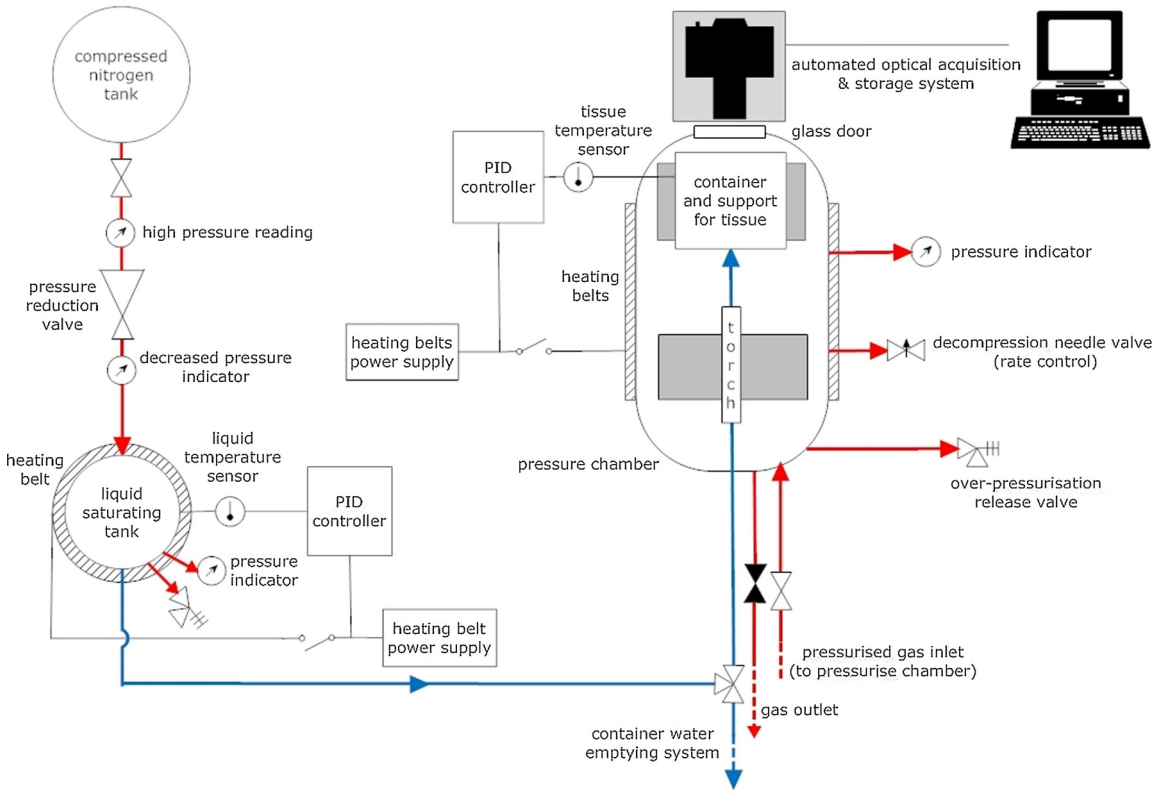

Fig. 1. Top view schematic of experimental set-up, showing liquid (in blue) and gas (in red) pressure flow systems. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)